Yeti training

Begin by explaining the fluidics.

- The containers with blue lids should be filled to the fill line with non-sterile MilliQ water. Spare water can be found in the large bottles in the box next to the Yeti.
- The containers with the red lids are the waste containers and should be emptied down the sink with plenty of water. Before placing back the waste containers back onto the machine again they should add a small amount of Chemgene to each waste tank (this can be found next to the lab sink).
- There are two containers at the bottom of the fluidics compartment. One of these is the Yeti's on-board cleaner (2% Decon). Check the levels of this and top up if necessary (a bottle of 2% Decon can be found on the lab bench next to the Yeti).
- The other container is sheath additive which helps to stop bacteria and mould growing in the sheath lines. Check the levels of this and replace with a new bottle if necessary (spare bottles of sheath additive can be found on the shelf behind the Yeti).

Then explain how to turn on the machine.

- Most of the time the machine and computer are on (blue light is visible at the front of the Yeti). In this case start the Everest software and type in the username and password ('xxx and 'xxx). If the machine if off there is a rocker switch on the back of the machine next to the power cable that needs to be turned on. If the computer also needs to be turned on then the password is 'xxx. Note that if the machine is off then even if the computer is on the Everest software will need to be restarted in order for it to connect to the machine.

Explain the fluidics startup.

- Click on the 'Startup' icon. Start-up should take approximately 5 minutes. The instrument status should change to 'Ready'.

Explain QC.

- QC should be run following start-up unless this has already been run that week (QC only needs to be run once a week). Show them the QC bead bottle on the machine. Explain that if they see no events the most likely cause is that the QC beads have run out. In this case they should find more in the fridge. Show them that when they change the QC bead bottle they will need to use the 'Change beads' icon on the Home screen to reset the bead volume to full (and change the lot number if necessary).

Explain how to set up a new experiment.

- From the Home window they should choose 'New Experiment' but explain that you can also load or edit a previous experiment from the list on the right hand side. Remind them that only experiments from the last month will be kept (just like our other analysers). All the rest are deleted and they should save either the run list file or the experiment if they wish to reuse old experiments. To use an old experiment and edit it they should use the 'Load Experiment' function and find their experiment file. Another option is to load the settings from a previous experiment into a new experiment. To do this they should use the 'Import Settings' function and find the relevant run list (RLST) file.
- Explain how to pick the fluorochromes and enter any axis labels they would like. The usual convention applies (filter, laser, fluorophore, target).
- Go through selecting a media type (plates/tubes. Show them the loader platform and tube racks, picking out which one to use for tubes versus which one for Eppendorfs.

The Experiment Setup screen should be explained in detail.

- Explain how they can set up wells/tubes as samples, wash, or setup. If they are running a lot of wells/tubes or if they are using clumpy samples such as tumour tissues or cell lines they should include wash wells/tubes. Use at least 1 well of 2% Decon followed by at least 1 well of water.
- For samples make sure that before they apply any settings they select all the samples they want the settings to apply to. Drag and drop can be used to order the wells/tubes to their preference.
- They should use this screen to set any settings they want to apply their tube rack/plate including the order in which they want the tubes/wells to run.
- They should put agitation on at least once per row of samples. If they are running large cell lines or clumpy samples it is worth adding agitation to every sample.
- Explain that if they are setting the loader to a temperature lower than room temperature then they should ensure that they put the temperature back up once their run is finished.
- Explain that they should always keep the wash on normal and that the flow rate they select should reflect how concentrated their samples are. Low or medium should be sufficient for most samples (medium is equivalent to running on high on the Fortessa).
- They should use the sample naming panel to enter the sample names. Names can also be imported from a spreadsheet with two columns (column 1 = well ID, column 2 = sample name).
- Explain that they should be careful when setting acquisition limits. Acquisition will stop when it reaches the first limit that has been set. When they set a limit they should press 'Enter' to apply it. Always set the volume limit to the volume in your tube/well if they want the event limit to apply. Remind them that the estimated run time is based on the volume limit so may not be accurate if they are recording a certain number of events per well/tube. Explain that if they want to set a gate limit they should only tick the tick box in this screen (without entering a value) and then set a gate and a limit in the following screen.
- Explain the 'Return Sample' setting and how it reverses the pump to return any unused sample once the event limit has been reached. Explain they if they want to run samples in high throughput mode they can only set a volume limit. In this mode samples are aspirated constantly and separated by a series of water and air boundaries. An event limit can only be set by running in standard mode.
- Explain that in order to use volumetric counting they will need to run the experiment twice. Volumetric counting takes up 10ul of sample once the flow is stabilised. So they should run this

before their main experimental run. They cannot run volumetric counting on a sample followed by acquisition.

- Explain that the 'Pause After' setting is most useful if they are running compensation and want to calculate it in the Everest software.
- Before they move away from this page they should use the double ended arrow to check their runlist and the settings they have applied to each tube/well.

Explain how to set voltages.

- Explain that the Yeti does not process negative numbers in the same way as the Fortessa will so the rule for setting voltages is slightly different. They need to run their fully stained sample first to get some approximate voltages. They should then run their unstained and make sure at least 90% of their negatives are on scale. From here they should just ensure their positive populations are also on scale.

Explain how to do compensation in the Everest software if this is what they plan to do.

- They will need to determine if beads or cells is best for their particular experiment. If they are using a combination of both they will need to either find scatter settings that accommodate both or run all the beads first then change the scatter voltages and run the cells.
- Remind them that (just like on the sorters) eBioscience beads do not work well on the Yeti.
- When running the single colours in the compensation template they should look out for populations that are above the diagonal line – this is where compensation will be over 100%. They should try and adjust voltages accordingly but remind them that they shouldn't worry too much about the values in the matrix. The compensation values can sometimes appear higher on the Yeti as it doesn't have the bi-exponential scale so there is less room to get everything on scale.
- Explain they should click on the 'Analyse' icon to calculate compensation once they have run all their controls (in acquisition mode with a pause applied to the last sample). The matrix icon can be used to calculate it. Open the matrix and explain that the traffic light system indicates if the control is good (green) or bad (red). Often if it is bad then it means there is insufficient resolution between the negative and positive populations or not enough of a positive population to calculate accurately. They can get around this either by running more of the control, replacing the marker with something more abundant (non tandem-dyes only), or by using beads instead of cells.
- To apply compensation they must click on 'Send to Instrument', this takes them back to the Acquisition part of the software and then compensation will be applied to their samples.
- Remind them that they must select the compensated parameters when they draw their plots to see the compensated data.
- Explain that they don't have to calculate the compensation in Everest; they can do it post-acquisition in FlowJo.

Explain how to create plots and regions for analysis.

- Explain that they need to pick parameters for the x axis before they can pick the y axis and that they can adjust the plot properties after it is created using the 'Plot Settings' icon. Show them how to bring up a statistics table, draw a region, and apply filters to a plot.
- Explain how the population hierarchy appears on the top of the plots rather than as a tree. Explain how to apply the gate limit (hand symbol). Explain that until they click 'Apply' the experiment will not be saved.

Explain how to run samples in Setup and Acquisition modes.

- Explain that in this mode they can record each sample but that any limits they set will not apply. Explain that before they start running samples in Setup mode (e.g. in order to set voltages) then they should reduce the flow rate down to 0.2µl/sec or less (otherwise the risk of them losing the sample is quite high – it gets aspirated quite quickly especially when they can't see what is happening). Explain how to change the voltage either by typing a value into the box, using the scroll wheel on the mouse, or by using the slider bars next to the detector name. Explain that if they click the cycle checkbox then their data will update as they change voltages.
- Explain that they should run samples in Acquisition mode when they are ready. Note that if their limits are wrong and the probe tries to suck up air it won't necessarily detect this and move to the next sample so until they have run a couple of wells they shouldn't walk away and leave the machine to it. Explain that they can use the arrow to skip over samples if necessary.

Explain how they can export their data.

- Explain that they should use the 'Export Data' icon in the Tools section of the toolbar. They should make sure that the tick box next to 'convert data for third party analysis' is unticked unless they are looking at data in Cytobank. They should export their files to the 'Yeti User FCS Files' folder on the desktop. They should then copy this to either facsscratch or NextCloud.
- Explain that if they forget to export their data then the FCS files are still saved and these can be found in the C drive under Program Data. Remind them that all experiments (and FCS files in this folder) will be removed after 1 month unless they specify that they will need to be kept for future use.
- If they wish to re-run the same experiment they should save the experiment. If they wish to save the settings for the experiment in order to re-use them in a new experiment they should save the run list (RLST) file.

Explain how to clean the machine and how to use the Pause System function.

- Stress the importance of using the 'Pause System' function given the limited supply of water to run the machine.
- Explain that they should run cleaning after every run and during runs if they are using a lot of samples or have clumpy samples. Cleaning should be run using the Stat Tube function in the Home window. 2% Decon can be pipetted into a 5ml tube for cleaning.
- Show them that there is also an automated sample line and probe clean cycle ('Clean ') which uses the on-board cleaner that they should use.
- Explain that in cases of blockages they should run 2% Decon for as long as they can. They should also run a few 'Unclog' cycles. This is found in the System part of the toolbar and is automated. After this the QC should be run to check that the unclog has worked.

Explain the shutdown of the Yeti.

- Explain that if they are last user of the day they can shut down the machine. They should make sure that there are enough fluids left to carry out the shutdown.